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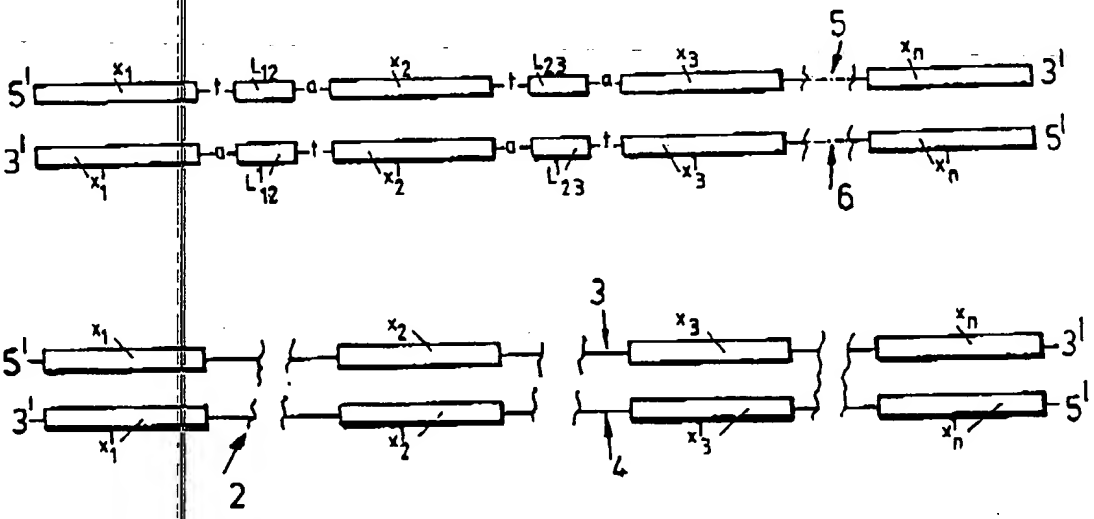

(57) Abstract

A method of producing a hybrid DNA molecule allowing the assembly of sequences x_1, x_2.....x_n where n is greater than or equal to 3 (e.g. give sequences) from diverse locations into a hybrid molecule for the purpose of mutation analysis. The method comprising the steps of: (1) providing in a single reaction mixture: (a) the sequences x_1, x_2.....x_n and their complementary sequences x_1', x_2'.....x_n', to be assembled into the hybrid molecule; (b) for each pair of complementary sequences defined in (a) a respective pair of PCR primers each having a priming sequence and which are such that the primers hybridising to the 3' ends of any two sequences (x_i, $x'_{(i+1)}$), where i is 1 to (n–1), have specifically complementary linker sequences: (2) effecting a first stage PCR reaction in which those primers provided with linker sequences are present in limiting concentrations; and (3) effecting a second stage PCR reaction using a single pair of primers one of which provides the 5'-end of the sense strand and other of which provides the 3'-end of the anti-sense strand of the required hybrid molecule; whereby said hybrid molecule is generated.